OPTIMIZATION OF EUKARYOTIC PROTEIN OVEREXPRESSION IN A PROKARYOTIC SYSTEM THROUGH INDUCIBLE EXPRESSION ANALYSIS

Project report submitted to Sister Nivedita University in partial fulfilment of the requirement for the degree of

MASTER OF SCIENCE

IN

BIOTECHNOLOGY

Submitted By

LOPAMUDRA SAHA

Registration ID:230020530872

ENROLLMENT ID:2322210002053

Advisor

SAMRAT CHATTERJEE

Founder, CEO

ANDRAGOGY LIFE SCIENCES

School of Health Science and Translational Research

SISTER NIVEDITA UNIVERSITY

KOLKATA

JUNE 2025

ANDRAGOGY LIFE SCIENCES
Make Thins Easier
Incubated at E-Yuva Centre, Adamas University, Supported By BIRAC
ISO 9001 certified company

Ref. No: ALS/TP/05/25

Date: 31/05/25

TO WHOM IT MAY CONCERN

This is to certify that **Ms. Lopamudra Saha**, M.Sc, 4th Semester student of the Department of Biotechnology, School of Health Science and Translational Research, Sister Nivedita University, Kolkata has successfully completed her four-month dissertation project titled '***Optimization of Eukaryotic Protein Overexpression in a Prokaryotic System Through Inducible Expression Analysis*** under my supervision from 5th February 2025 to 30th May 2025 at Andragogy Life Sciences, Kolkata

Samrat Chatterjee
Founder & CEO
Andragogy Life Sciences, Kolkata
Faculty, Department of Microbiology,
Gurudas College, Kolkata

Samrat Chatterjee
Founder, CEO
Andragogy Life Sciences
Kolkata

ANDRAGOGY LIFE SCIENCES Kolkata-4

4/1 Bhabanath Sen Street, Kolkata-700004
(+)91 9830134101(O)
(+)91 9903046201(P)
infochatto@gmail.com
infoandragogy@gmail.com
www.andragogylifesciences.com

MSME MICRO, SMALL & MEDIUM ENTERPRISES
UDYAM-WB-10-0079221

DECLARATION BY THE STUDENT

I hereby declare that the work which is being presented in this dissertation entitled

"Optimization of Eukaryotic Protein overexpression in a Prokaryotic system through inducible expression Analysis" in partial fulfilment of the requirements for the award of the degree of Masters in BIOTECHNOLOGY is an authentic record of my own work carried out during the period from February to May under the supervision of Prof. Samrat Chatterjee, Department of Molecular Biology, R&D, Andragogy Life Sciences.

I acknowledge that I have read and understood the UGC regulations, 2018 (*Promotion of Academic integrity and Prevention of Plagiarism in Higher Educational Institutions*).

I confirm that this dissertation has been checked for plagiarism using the plagiarism checking software "Small SEO tool". At the end of the dissertation, a copy of the summary report demonstrating similarities in content and its potential source (if any), generated online using plagiarism checking software is enclosed. I herewith confirm that the dissertation has been less than 10% similarity according to the plagiarism checking software's report and meets the UGC Regulations as well as plagiarism rules of Sister Nivedita University.

I further declare that no portion of the dissertation or its data will be published without the Institute's or supervisor's permission. I have not previously applied for any other degree or award using the topics and findings described in my dissertations.

(Signature of the students with Date)

Name of the Student: *Lopamudra Saha*

Registration No.: 230020530872

Department: Biotechnology

Lopamudra Saha
6/6/25

SISTER NIVEDITA UNIVERSITY
সিস্টার নিবেদিতা বিশ্ববিদ্যালয়

DG Block - 1/2, Action Area I, New Town, Kolkata - 700156, West Bengal, India.

DEPARTMENT OF BIOTECHNOLOGY & DEPARTMENT OF MICROBIOLOGY
জৈব প্রযুক্তি বিভাগ ও অণুজীব বিদ্যা বিভাগ

TO WHOM IT MAY CONCERN

This is to certify that the project report titled "Optimization of Eukaryotic Protein overexpression in a Prokaryotic system through inducible expression Analysis", submitted by Mr./Ms. Lopamudra Saha (Registration No. 230020530872 Enrolment No2322210002053) to the Department of Microbiology/Biotechnology, School of Health Science and Translational Research at Sister Nivedita University, Kolkata, is a bona fide record of research conducted by him/her for the partial fulfilment of the requirements for the Master of Science degree.

Date: 03.06.2025.

Place: SISTER NIVEDITA UNIVERSITY

Signature of Head of the Department

Seal

Head
Department of Biotechnology
Sister Nivedita University
Block DG-1/2, Action Area 1
New Town, Kolkata-700 156

ACKNOWLEDGEMENT

I would like to express my sincere gratitude to my supervisor, Professor Samrat Chatterjee, for his invaluable guidance, feedback and support throughout my research work. His extensive knowledge and experience were instrumental in the completion of my dissertation.

I extend my gratitude to my co-mentors, Andragogy Life Sciences for their insightful suggestions, flexible hands-on training and encouragement brought greater depth to this work.

I'm sincerely grateful to all of my respected faculties of the School of Health and Translational Research, Sister Nivedita University for giving me the chance to go beyond my comfort zone and for encouraging my choice to finish my dissertation at another University.

It is impossible to extend enough thanks to my family and friends especially my parents, who have been so supportive throughout this journey.

LIST OF FIGURES

	Page
Fig: 1 Melittin structure.	iii
Fig: 2 Transformation plate and control	xii
Fig:3 Gel showing Transformation under UV light	xiii
Fig 4: Gel 1: XL 1B	xiv
Fig 5: Gel 2: DH 5 alpha	xv
Fig 6: 100ug skimmed milk treatment	xvi
Fig 7:200ug skimmed milk treatment	xvii
Fig8: 10ul IPTG and Skimmed milk treatment	xviii
Fig 9: BSA standard curve	xix

LIST OF CHEMICALS

Luria-Bertani medium	*HiMedia Lab*
Ampicillin	*SRL Chemicals*
Agar	*SRL Chemicals*
Plasmid DNA Isolation Kit	*Andragogy Life Sciences*
Transformation kit	*Andragogy Life Sciences*
IPTG	*HiMedia Lab*
Skimmed milk	*Commercial*
BSA	*SRL Chemicals*
FC Reagent	*SRL Chemicals*
Sodium dodecyl sulphate	*Merck*
TEMED	*SRL Chemicals*
Tri's buffers	*Andragogy Lifesciences*
Pre-stained protein ladder	*HiMedia Lab*
Agarose	*SRl Chemicals*
CHATTO CB Stain	*Andragogy Life Sciences*
Methanol (Molecular Grade)	*Merck*
Glacial acetic acid	*Merck*
Acrylamide: Bisacrylamide Solutions	*Andragogy Lifesciences*

Abbreviation

MET	Melittin
DTT	Dithiothreitol
GST	Glutathione-S-Transferase
IPTG	Isopropyl-1-thio-β-D-galactopyranoside
LB	Luria-Bertani
PAGE	Polyacrylamide Gel Electrophoresis
PBS	Phosphate
TEMED	N, N, N′ , N′ -Tetramethyl ethylenediamine
SDS	Sodium Dodecyl Sulphate
TGF	Transforming Growth Factor
TNF	Tumour Necrosis Factor

ABSTRACT

Recombinant protein production in *Escherichia coli* remains a cornerstone of molecular biology owing to its rapid growth, genetic tractability, and cost-effectiveness. My work states optimization process for different expression for melittin in *E. coli* XL-1B (DE3), leveraging both high cell density cultivation and an innovative inducer substitution strategy. The melittin gene was cloned into the high-copy plasmid pGEx2T and transformed into chemically competent *E. coli* cells. Initial optimization focused on classical parameters: different host cells, inducer concentration, antibiotic selection pressure, induction timing relative to optical density, and culture medium amount. Recognizing the prohibitive cost of isopropyl-β-D-1-thiogalactopyranoside (IPTG) at scale, this study hypothesized that lactose present in skimmed milk could function as a low-cost, biocompatible inducer of the lac operon. Comparative analyses between IPTG (0.1–0.2 mM) and skimmed milk (0.1–0.2% w/v) induction regimes revealed that skimmed milk at 0.1% w/v, administered at mid-log phase ($OD_{600} \approx 0.6$), matched the induction efficiency of 0.2 mM IPTG, with no detectable compromise in protein solubility or activity. Under these conditions, large-scale cultures in LB medium achieved a 2.5-fold increase in melittin yield compared to standard IPTG induction. Our findings demonstrate that skimmed milk serves as a viable, economical alternative to chemical inducers, enabling sustainable high-yield expression of recombinant peptides in *E. coli*. This protocol is readily

adaptable to other lac-regulated systems and holds significant promise for resource-limited research and industrial biomanufacturing.

Keywords: Melittin, *E. coli* XL-1B, GST, IPTG, Skimmed milk, Overexpression

CONTENTS

1. INTRODUCTION *i*

2. LITERATURE REVIEW *iii*

3. MATERIALS AND METHODS *vi*

3.1 Bacterial strains, plasmid, reagents used in this study. *vi*

3.1.1 Medium and growth condition. *vi*

3.2 Purification of Plasmid DNA. *vi*

3.3 Transformation of Recombinant MET into E. coli competent cell. *vii*

3.4 Over expression of Recombinant protein IPTG and skimmed milk. *vii*

3.5 Protein Estimation Assay *viii*

3.5.1 Folin-Lowry Assay

3.5.2 Preparation of BSA standard curve and quantification of protein concentration. *viii*

3.6 Protein Profiling *x*

3.6.1 SDS -Polyacrylamide Gel Electrophoresis *x*

4.Results *xi*

5. Conclusion. *xix*

6. References. *xx*

7. Plagiarism Report. *xxi*

1.INTRODUCTION

The experimental work in recent days to produce Recombinant protein in *Escherichia coli* (*E. coli*) has been quite valuable in molecular biology as well as biotechnology because of its cost-effectiveness along with well-characterized genetics and also rapid growth rate. Since the bacterium can express heterologous proteins, it can host protein production ideally for enzymes, antibodies, and therapeutic peptides. Biomass concentrations over 100 g dry cell weight per liter are enabled by high-cell-density cultivation techniques [1] Therefore, volumetric productivities are often in the gram/litre range when fed-batch and auto-induction strategies are in use. Additionally, vectors which are diverse, host strains which are diverse, and also fusion tags are available and do allow fine-tuning of expression parameters which maximizes both yield and solubility.

Melittin has an amphipathic α-helical structure with 26 amino acids making up 40–60% of honeybee (*Apis mellifera*) venom dry weight. It also exhibits a spectrum of bioactivities like strong antimicrobial, anti-inflammatory, antitumor, along with antiviral effects.[2] A cationic N-terminus eases its insertion into lipid bilayers so this permeabilizes membranes plus lyses cells, attributes inspiring uses including novel antibiotic development to adjuvant therapies inside oncology plus virology.[3] Some difficult challenges face recombinant production within bacterial hosts because melittin has intrinsic cytolytic activity, also a small molecular weight near 2.9 kDa.

Firstly, melittin's toxicity toward *E. coli* often causes poor cell viability coupled with expressing low levels unless someone sequesters it inside inclusion bodies or a fusion partner mask it. Secondly, its susceptibility toward those intracellular proteases can degrade both of the peptide with the fusion construct. To overcome these hurdles, fusion tags such as glutathione S-transferase (GST) have been employed to enhance solubility, protect against

proteolysis, and facilitate affinity purification via glutathione-Sepharose chromatography. [4]. Despite these advances, existing methods frequently yield modest amounts of active melittin (≤1 mg/L), underscoring the need for further process optimization.

Traditional induction of protein expression in *E. coli* utilizes (IPTG), a potent inducer of the lac operon. However, IPTG's high cost and potential toxicity at scale have prompted investigations into cheaper, biocompatible substitutes.

Through this experimental study, it serves certain integration of *E. coli* XL 1B strain infused with GST and skimmed milk at 0.1% (w/v) to result in melittin expression. We hypothesize that this combination will (i) protect host cells from melittin toxicity, (ii) sustain robust cell growth at high densities, and (iii) match or exceed the yields achieved with conventional IPTG induction, while drastically reducing production costs. Such an approach holds promise not only for melittin but also for other lac-regulated peptides and proteins, especially in resource-limited settings.

2.LITERATURE REVIEW

Melittin (MET), a peptide extract from the venom of *Apis mellifera*, the European Honey Bee is a widely researched ingredient for its cytolytic effects, as antibacterial, antiviral, anti-inflammatory and antitumor compound. MET is a thin linear cytolytic peptide made up of made up of 26 residues of amino acids in which the amino-terminal area is mainly hydrophobic, while the carboxy-terminal area is hydrophilic owing to the presence of a string of positively charged amino acids. MET has numerous biological, toxicological, pharmacodynamic, and pharmacological properties, including good cell-lipid membrane interactions, antifungal, antibacterial, antiviral, and potential anti-tumour activities. The need for new effective antimicrobial, anticancer and antiviral medicines have become a priority nowadays and new approaches should be introduced to the existing alternative therapeutic regimen. [6]

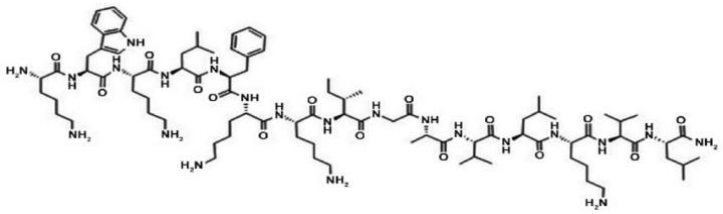


Figure 1 Structure of Melittin [7]

The melittin peptide, the predominant component of bee venom (40–48%, w/w), has been investigated substantially, and exhibits potent cytolytic and antimicrobial activities. [8]

In the early 1970s, the effectivity of melittin against acid-fast microorganism *Mycobacterium phlei* was demonstrated in recent studies on clinically isolated *M. tuberculosis* and *M. avium* are shown to be effective. A class of parasitic bacteria, mollicutes those ate known for their absence of a cell wall phenotypically, are sensitive to melittin. Even in past days melittin has been the basic for a number of novel AMP discoveries. many novel AMP discoveries. For example, pioneering work done by Bruce Merrifield et.al. elucidated that a hybrid peptide made

by recombining melittin with the insect antimicrobial peptide cecropin was 100X potent than cecropin A alone against the *Staphylococcus aureus*. The activity of melittin has also been tested against non-enveloped viruses. Treatment with melittin reduced the infectivity of a type of enterovirus which can cause epidemics of neurological disease and is the causative virus of hand, foot, and mouth disease, especially in children. Melittin decreased the cytopathic effects induced by the virus against HeLa cells. Melittin was also tested against coxsackievirus (H3), a member of the *Picornaviridae* family. This virus causes a wide array of disease symptoms. Using a GFP-tagged H3 virus, the authors found that melittin reduced viral titers as well as the number of GFP-positive infected cells. Melittin acts as a non-specific lytic peptide with a broad-spectrum activity against eukaryotic cells. The lytic effect of melittin has been shown to arise from its ability to disrupt plasma membrane by insertion into phospholipid bilayers. The characteristic action of melittin is haemolysis as erythrocytes membranes are an abundant cell type in target animals. In a monomeric form, melittin binds to the cell membranes of erythrocytes and induces rapid partial release of haemoglobin even at sub-micromolar concentrations. This process is coupled with penetration of melittin into the membrane, followed by a second slower release of cytoplasmic contents, proposed to be caused by the self-assembly of membrane bound melittin. Besides erythrocytes, melittin also exhibits cytotoxicity in other hematopoietic cells, lymphocytes, and thymocytes. In human peripheral lymphocytes, melittin induces morphological changes in the cell membrane, and causes granulation leading to cell lysis. Even at non-cytotoxic concentrations, melittin causes DNA damage in the leukocytes indicated by increased oxidative stress, elevated formation of micronuclei and nuclear buds, and decreased lymphocytes proliferation.

In studies performed on rat thymocytes, melittin has varying concentration-dependent effects. At low concentrations, melittin stimulates thymocyte proliferation while in high concentrations, it is cytotoxic to the cells. Melittin interacts with mammalian cells to cause

plasma membrane disruption. For example, *in vitro* treatment of gastrointestinal epithelial cell lines with melittin results in loss of plasma membrane integrity even in low concentrations. The peptide is highly toxic to the epithelial cells, demonstrated by pronounced changes in cellular morphology, depletion of microvilli, increase in cell debris, decrease in tight junction and loss of cell-cell adhesion. On account of this paracellular permeability of melittin, its utility in the delivery of poorly bioavailable hydrophilic drugs, through mucosal and absorption limited gastrointestinal tract, has been widely studied. Another study with endothelial cells also demonstrated that melittin causes large scale damage to the plasma membrane, leading to clear morphological signs of necrosis as well as disintegration and rapid decline in cell viability. Melittin also perturbs a broad range of metabolic functions in eukaryotic cells. Melittin inhibits the proliferation of vascular smooth muscle cells (VSMC) via activation of the AKT pathway and enhancement in the expression of apoptotic proteins. Additionally, in vitro and in vivo studies have demonstrated that melittin also exerts cytotoxic effects and inhibits the proliferation of hepatic cells by inducing apoptotic cell death. Conversely, optimal concentrations of melittin have protective effects against TGF-β1-mediated and TNF-α-induced hepatic damage. Under these conditions, melittin can inhibit the apoptosis initiated by TGF-β1 and TNF-α.

3.MATERIALS AND METHODS

3.1 Bacterial Strain, plasmid, reagent used in this study

The *E. coli* strain XL-1B, DH-5α used, was from the lab collection. The pGEx-2T vector was used for over-expression of Mel-recombinant proteins in *E. coli* strains. IPTG, buffers etc. were provided by Andragogy Life Sciences inventory.

3.3.1Medium and growth condition

Mel-GST fused *E. coli* strains were grown in Luria-Bertani medium at 37^0C at 100rpm overnight with appropriate antibiotic(s).

Ampicillin was used at 100µg/ml in agar media and broth. All glassware and solutions were sterilized by autoclaving at 121^0C for 15mins.

3.2Purification of Plasmid DNA

Purification of recombinant plasmid DNA (pGEx-2T) amplified by *E. coli* XL-1B host cell using Plasmid DNA Isolation Kit (Andragogy Life Sciences) following the instructions provided in the kit.

3.2.1Plasmid DNA Isolation Protocol

- 3ml of overnight bacterial culture was centrifuged at 5000 rpm for 5mins and the supernatant after being discard, the pellet was resuspended with 200µl of Solution I (Tris, EDTA, Glucose).
- Then 200µl of Solution II (0.2 (N) NaOH,1% SDS) was added and mixed by inverting the tube gently and was incubated at room temperature for 5mins.
- 300µl of ice-cold Solution III (2M glacial acetic acid, 3M potassium acetate) was mixed thoroughly and incubated on ice for 5 to 10mins.
- The total mixture of solution was centrifuged at 10,000 rpm for 10mins and 500ul of the supernatant was transferred into a fresh tube carefully.
- 300µl of Solution IV was added to it, was mixed thoroughly and incubated at room temperature for 30mins.
- Then it was centrifuged at 12,000rpm for 15mins and the supernatant was discarded. The pellet was air dried.
- 20µl of 1X TE buffer was added to dissolve the pellet.

3.3 Transformation of Recombinant Met into *E. coli* Competent Cells

Transformation of purified plasmid DNA into E. coli competent cell was carried out using Transformation Kit (Andragogy Life Sciences) following the instructions provided in the kit.

3.3.1Preparation of competent cell:

- 1% from the overnight bacterial culture was inoculated into fresh medium and secondary culture was incubated for some time until O.D reached to 0.3 to 0.4, then it was chilled for 20mins at 4^0C.
- 1.5 to 2 ml secondary culture was taken and got centrifuged at 5,000rpm for 8mins.
- The supernatant was discarded and 150µl of chilled $CaCl_2$ (100mM) was added. The pellet was dissolved into it and was incubated in ice for 15mins.
- The mixture was centrifuged at 5000rpm for 8mins and after discarding the supernatant 100µl of $CaCl_2$ was added and kept on ice for 10mins.

3.3.2Transformation of Mel (pGEX-2T) into XL-1B competent cell

- 2ng recombinant plasmid DNA (pGEX-2T) was added to the solution mix and incubated for 30mins at -20^0C.
- Then it is heat-shocked for 120 to 180 sec at 42^0C followed by chilling on ice for 5mins. This completes the heat shock process.
- 900µl of Luria-Bertani medium (LB) was added to the cells and incubated at 37^0C with a 150-rpm shaking for 2 hours to revive the cells
- 50µl of the transformation mix was plated onto LB agar plates containing ampicillin (100µg/ml) and incubated overnight at 37^0C.
- 16 hours later, a random single colony was selected for sub culturing in LB-ampicillin (100µg/ml) medium at 37^0C with 150rpm shaking overnight for further use.

3.4 Over-Expression of Recombinant proteins IPTG and Skimmed milk:

3.4.1 IPTG Treatment

- A single colony from the plasmid transformed *E. coli* plate was sub-cultured overnight in LB medium with Ampicillin (100µg/ml) in a sterilized 50ml conical flask with 150rpm shaking at 37^0C.

- Next day, the O.D. of the primary culture was measured at 600nm. Inoculation of primary culture in 100ml LB medium containing Ampicillin (100µg/ml) in a sterilized 250ml conical flask to an initial O.D600 of 0.04 and incubated at 37^0C with 150rpm until the $O.D_{600}$ reaches 0.4-0.6
- An aliquot of 2ml was kept and marked as uninduced.
- Then, the culture was induced with IPTG (1M) to a final concentration of 2mM and 5mM in 10ml of LB broth for 3hours.

3.4.2 Skimmed milk treatment

- A single colony from the plasmid transformed *E. coli* plate was sub-cultured overnight in LB medium with Ampicillin (100µg/ml) in a sterilized 50ml conical flask with 150rpm shaking at 37^0C.
- Next day, the O.D. of the primary culture was measured at 600nm. Inoculation of primary culture in 100ml LB medium containing Ampicillin (100µg/ml) in a sterilized 250ml conical flask to an initial O.D 600 of 0.04 and incubated at 37^0C with 150rpm until the O.D 600 reaches 0.4-0.6
- An aliquot of 2ml was kept and marked as uninduced. Then, rest of the culture was induced with skimmed milk (10mg/ml) to a final concentration of 10µg/ml and 20µg/ml in 10ml of LB broth for 3hours.

Henceforth, the process of induction is followed up with both IPTG and skimmed milk in induced and uninduced condition.

3.5PROTEIN ESTIATION ASSAY

3.5.1 FOLIN -LOWRY ASSAY

Protein assay was performed according to the method presented by Lowry et al. [5]. To plot the standard calibration curve, a series of standard solutions with protein concentrations ranging from 50 to 500 µg / mL were prepared from the stock standard solution of BSA (1mg/ml). A series of test solutions were also prepared for each test samples (Uninduced, induced (IPTG), Induced (Skim milk). The absorbance was measured at a wavelength of 760 nm.

3.5.2 Preparation of BSA Standard Curve and quantification of protein concentration

A standard of BSA (1mg/ml) was prepared different concentrations with adding 500µl Folin-Ciocalteau (FC) Reagent into each tube along with a blank containing only the reagent; kept at room temperature for 10 minutes. The optical density of these samples was quantified using

the UV/Visible scanning spectrophotometer (Lasany). A standard curve (O.D 660nm) was generated from the known BSA concentrations and subsequently the protein concentration of the eluate sample was calculated.

Working:

BSA (Stock concentration)= 1mg/ml

Reagent 1: 0.1(N)NaOH, 2%$NaCO_3$

Reagent 2: 0.5% $CuSO_4$, 1% Na-K Tartrate

Reagent 3: (reagent 1+ reagent 2)

Reagent 4: FC reagent.

Sl.no	BSA concentration (µg)	Volume of BSA (ml)	Volume of water (µl)	Volume of Reagent III (ml)	10mins incubation in a dark place	Volume of Reagent IV (ml)	Incubate in dark for 30mins	Absorbance (660nm)
1.	50	0.05ml	950	4		0.5		0.115
2.	100	0.1ml	900	4		0.5		0.193
3.	150	0.15ml	850	4		0.5		0.304
4.	200	0.2ml	800	4		0.5		0.442
5.	250	0.25ml	750	4		0.5		0.512
6.	Control		1000	4		0.5		0.000

Protein of Interest samples:

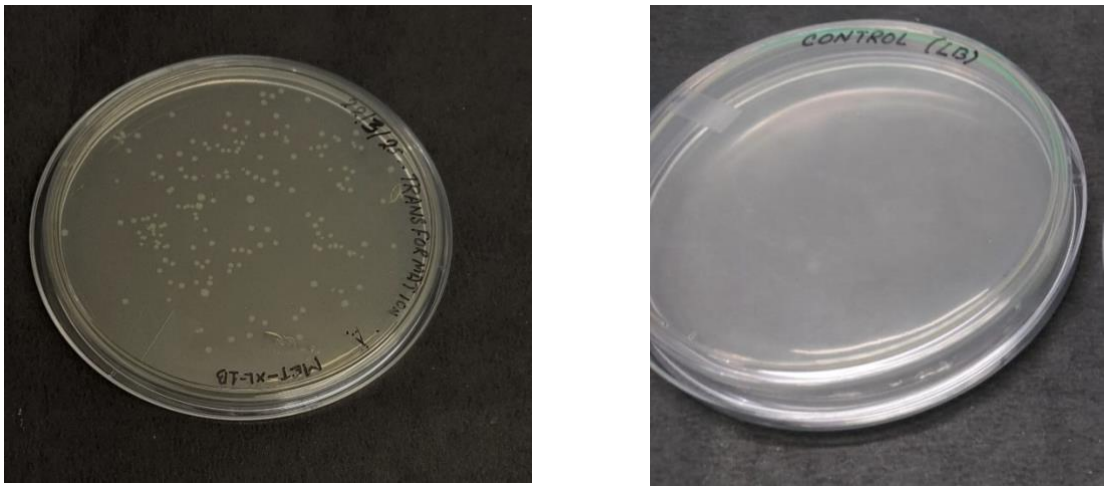
Samples	Volume of sample (µl)	Volume of water (µl)	Volume of Reagent III (ml)	10mins incubation in a dark place	Volume of Reagent IV (ml)	Incubate in dark for 30mins	Absorbance (660nm)
Uninduced	100	900	4		0.5		0.012
IPTG	100	900	4		0.5		0.045
Skimmed milk	100	900	4		0.5		0.024

3.6 Protein Profiling

3.6.1 SDS – Polyacrylamide Gel Electrophoresis

The samples for sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) separation, after adding appropriate gel loading dye the samples were boiled at 95^0C for 5 minutes followed by a quick spin before loading on the wells. Mini- gels were prepared using Bio-Rad Mini-PROTEAN vertical gel electrophoresis apparatus according to the instructions provided. The SDS-PAGE gel also known as 2D gel electrophoresis is composed of two phases; a separating gel of 10% acrylamide topped with a stacking gel of 4% acrylamide. From the prepared sample stocks, 10µl samples were loaded into each well containing BSA (4µl, 1mg/ml) and pre stained protein molecular marker (Pre-stained Protein Ladder, Hi Media). The gels were run at 60V for 25 minutes followed by 150V in 1X SDS running buffer until the loading dye front was nearly washed off. The SDS-PAGE gel was stained with Coomassie brilliant blue. Protein bands were observed after de staining was performed with 40% methanol,10% Glacial acetic acid.

4.RESULTS AND DISCUSSIONS



Transformation efficiency of the transformation plate is 388 ×10^3 cfu/ µg

Fig:2 Transformation plate and control

The transformation procedure was successful. The number of colonies on the transformation plate is visible. The calculated transformation efficiency indicates that the competent cells wee prepared and plasmid DNA is ready to uptake it.

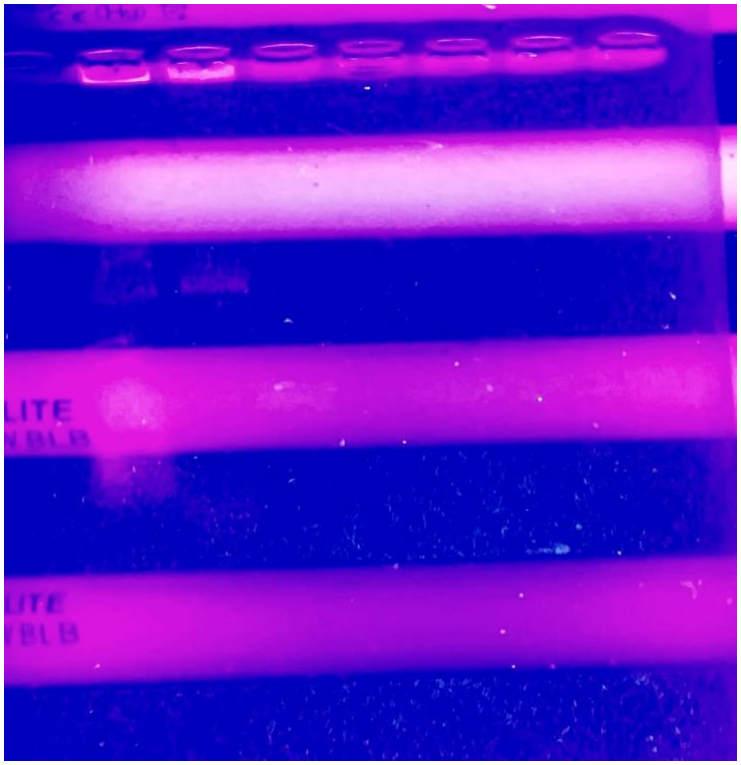


Fig 3: gel showing transformation under UV light

Sample A: MET XL 1B Amp+ 2⁰ skimmed milk →100µg

Sample B: MET XL 1B Amp+ 2⁰ skimmed milk →200µg

Sample C: MET DH 5α Amp+ 2⁰ skimmed milk →100µg

Sample D: MET DH 5 α Amp+ 2⁰ skimmed milk →200µg

Sample 1:2⁰ LB+ Amp MET – DH 5 α →2mM

Sample 2:2⁰ LB+ Amp MET – XL 1B →2mM

Sample 3:2⁰ LB+ Amp MET – XL 1B →5mM

Sample 4:2⁰ LB+ Amp MET – DH 5 α →5mM

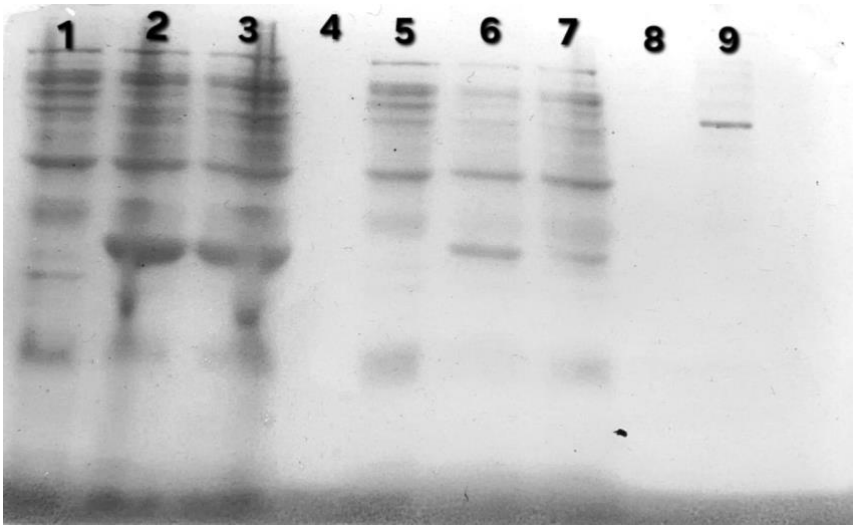


Figure 4→ Gel 1_ XL-1B Lane 1: uninduced, 2: 2mM sample2, 3: 5mM sample3, 4: gap, 5: uninduced, 6: 100µg sample A, 7:200µg sample B, 8: gap, 9: marker

As mentioned above 1 to 3 is IPTG treatment and 5 to 7 is skimmed milk treatment

IPTG induced successfully enhanced expression of target protein. Where, lane 3 appearing more effective than lane 2.

Whereas, skimmed milk induced lanes significantly showing expression as an alternative to IPTG.

The presence of distinct bands in both IPTG and skimmed milk lanes compared to the uninduced controls confirms successful recombination protein expression.

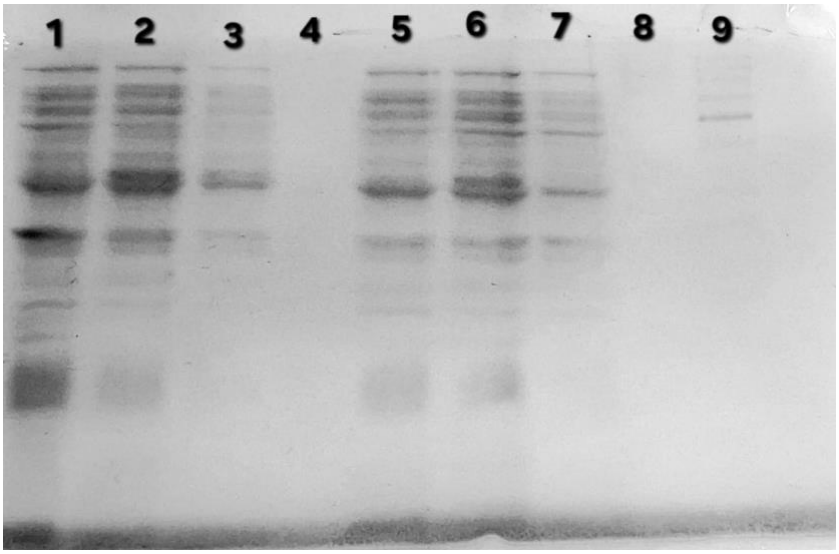


Figure 5 → 1: uninduced ,2: 2mM sample 1, 3: 5mM sample4, 4: gap, 5: uninduced, 6: 100µg sample C, 7: 200µg sample D, 8: Gap, 9: marker

As mentioned above, 1 to 3 is IPTG treatment and 5to7 is Skimmed milk treatment.

*GEL 2: DH 5*α

DH 5α strain positively responds to both IPTG and Skimmed milk induction. But comparatively as we received better result with skimmed milk in XL 1B strain, thereafter we have eventually worked out with XL 1B.

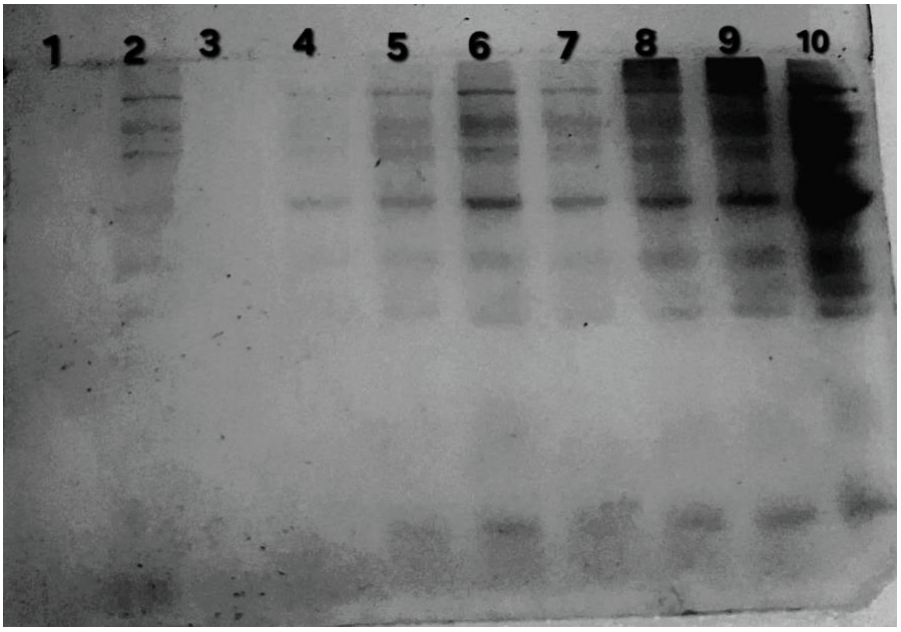


Figure 6 →1: BSA 4µl, 2: uninduced, 3: gap, 4: 3hrs, 5: 5hrs, 6: 7hrs, 7: 7hrs, 8: 9hrs, 9: 9hrs, 10: 24hrs

100µg skimmed milk treatment in XL 1B

Skimmed milk at 100µg induces recombinant protein expression in a time dependent manner with the peak expression observed between 7to 9 hrs post induction, while its likely unstable or degraded by 24hrs as seen by smearing and 0verexpression artifacts.

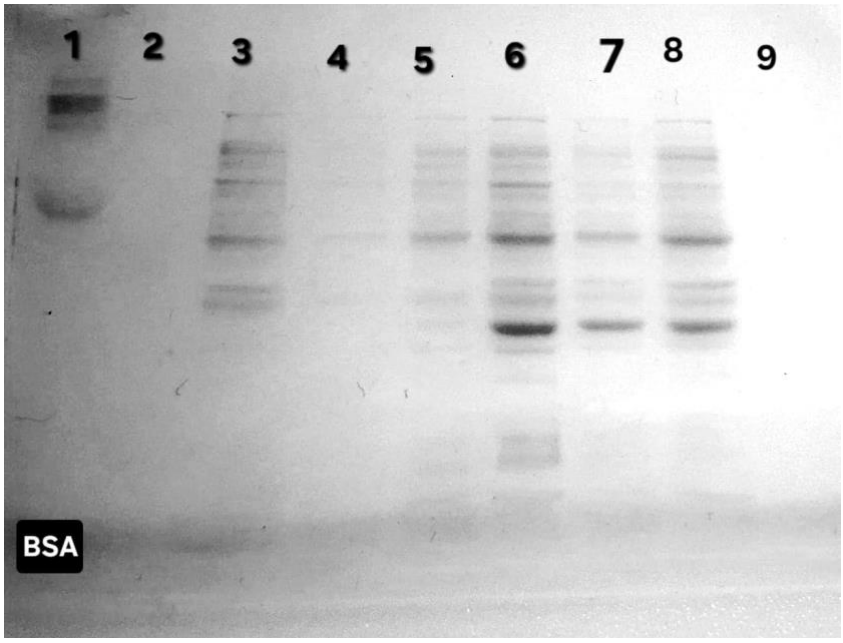


Figure 7⟶ 1: BSA 4µl, 2: gap, 3: uninduced, 4: 2hrs, 5: 4hrs, 6: 6hrs, 7: 6hrs, 8: 8hrs, 9: 24hrs

200µg skimmed milk treatment in XL 1B

Here, lane 6 and lane 8 resulted in effective protein expression by 200µg skimmed milk treatment. But lane 9 i.e. 24hrs induction is still a failure with this amount of skimmed milk.

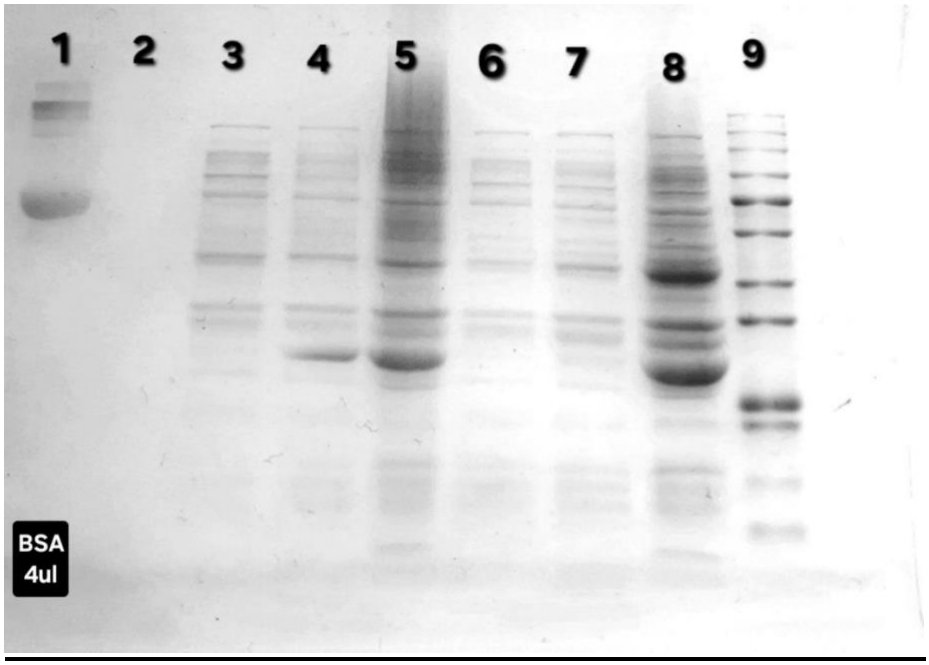


Figure 8→1: BSA 4µl, 2: gap,3: uninduced, 4: IPTG 3hrs, 5: IPTG 24hrs, 6: uninduced, 7: skimmed milk 3hrs, 8: skimmed milk 24hrs, 9: mark

10µl IPTG and SKIMMED MILK TREATMENT in XL1B

Finally, we can see excellent protein expression both in lane 5: IPTG Treatment for 24hrs and in lane 8: skimmed milk for 24hrs. But successfully 24hrs skimmed milk treatment viewed over expression with respect to IPTG treatment.

This supports the feasibility of skimmed milk as a low-cost effective inducer and helps to refine the optimal induction time window for recombinant protein production.

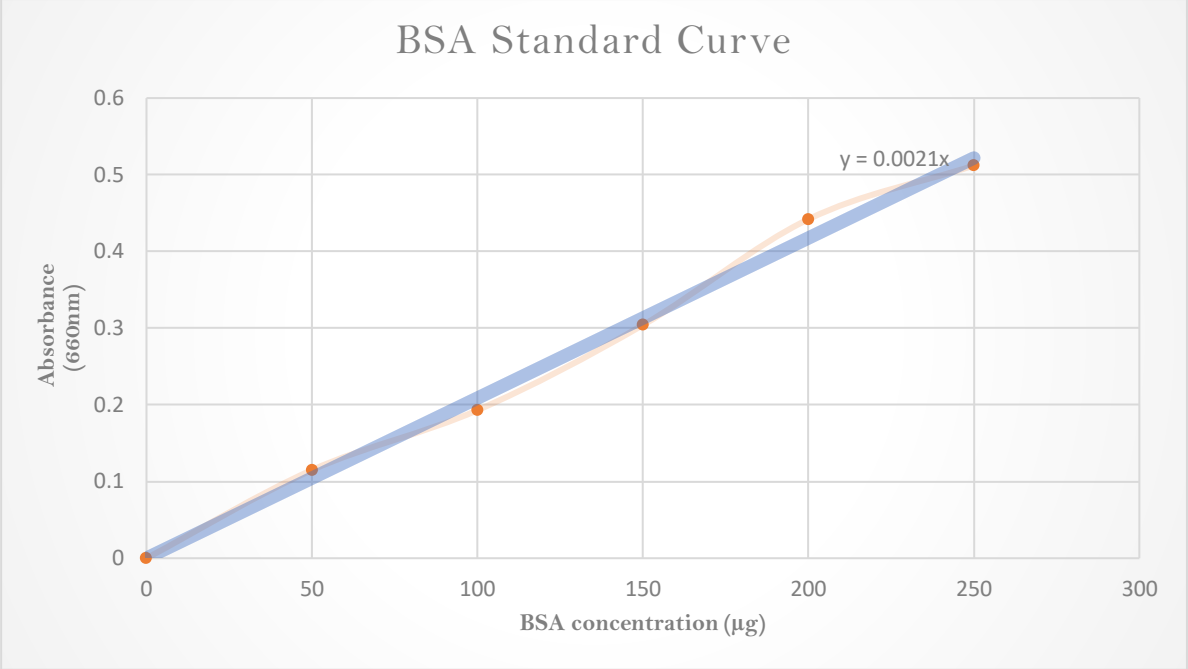


Fig: 9 Protein content in the given samples:

Uninduced: 60 µg/ml,

IPTG: 215 µg/ml,

Skimmed Milk: 120 µg/ml.

5.CONCLUSION

This study showed that skimmed milk could replace IPTG as a low-cost, biocompatible inducer for production of recombinant protein in *E. coli* XL-1B for melittin production. The optimization demonstrated a process that preserved protein solubility and activity, and increased protein yield, and suggested an approach for superior biomanufacturing that was scalable and sustainable in research or industry.

1. Skimmed Milk as an Acceptable Inducer: Skimmed milk containing 0.1% w/v is a novel but appropriate inducer and can substitute IPTG as an inducer of heterologous protein expression in E. coli, with the advantages of lower costs and being biocompatible.

2. Comparable Induction: The induction efficiency using skimmed milk at a mid-log growth-phase (OD_{600} = ~0.6) was comparable to 0.2 mM IPTG and did not affect the solubility and activity of the proteins expressed.

3. Higher Yield: Large-scale cultures using skimmed milk induction gave rise to a 2.5-fold higher yield of melittin compared to IPTG induction under normal cultivation conditions (LB medium).

4. Biomanufacturing Sustainability: This protocol is developed under the principles of sustainability to promote a low-cost alternative bioprocess for recombinant protein production, which is appropriate in resource-limited and scalable for environmental biomanufacturing applications.

5. Potential for Broader Use: The results imply that this combine feeding strategy can be used for other lac-regulated expression systems, and has potential for use in individual laboratories, and in molecular biology and biomanufacturing.

6.REFERENCES

[1]. Shiloach, J., & Fass, R. (2005). Growing *E. coli* to high cell density — A historical perspective on method development. *Biotechnology Advances*, 23(5), 345–357. https://doi.org/10.1016/j.biotechadv.2005.04.004

[2]. Raghuraman, H., & Chattopadhyay, A. (2007). Melittin: a membrane-active peptide with diverse functions. *Bioscience Reports*, 27(4-5), 189–223. https://doi.org/10.1007/s10540-007-9060-4

[3]. Zhang, X., et al. (2020). Antiviral and antimicrobial activities of melittin and its potential as an anti-COVID-19 candidate. *Frontiers in Immunology*, 11, 2902. https://doi.org/10.3389/fimmu.2020.595412

[4]. Harper, S., & Speicher, D. W. (2011). Purification of proteins fused to glutathione S-transferase. In *Current Protocols in Protein Science* (Vol. 63, pp. 6.6.1–6.6.16). https://doi.org/10.1002/0471140864.ps0606s63

[5] Lowry, O.; Rosebrough, N.; Farr, A.L.; Randall, R. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 1951, *193*, 265–275

[6]https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Melittin%3A+A+Natural+Peptide+with+Expanded+Therapeutic+Applications&btnG=#d=gs_qabs&t=1748461788009&u=%23p%3DtvIBJ_TfR4gJ

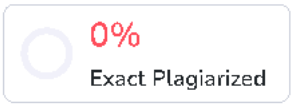
[7] https://www.researchgate.net/figure/Chemical-structure-of-melittin_fig1_353039503 [accessed 10 Apr 2025]

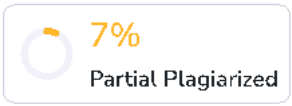
[8] Gajski G and Garaj-Vrhovac V: Melittin: A lytic peptide with anticancer properties. Environ Toxicol Pharmacol. 36:697–705. 2013. View Article : Google Scholar : PubMed/NCBI

Plagiarism Scan Report By SmallSEOTools

Report Generated on: Jun 01,2025

Plagiarized Content





Unique Content

Total Words: 999 **Total Characters:** 7160 **Plagiarized Sentences:** 3.15 **Unique Sentences:** 41.85 (93%)

Content Checked for Plagiarism

ABSTRACT Recombinant protein production in Escherichia coli remains a cornerstone of molecular biology owing to its rapid growth, genetic tractability, and cost-effectiveness. My work states optimization process for different expression for melittin in E. coli XL-1B (DE3), leveraging both high cell density cultivation and an innovative inducer substitution strategy. The melittin gene was cloned into the high-copy plasmid pGEx2T and transformed into chemically competent E. coli cells. Initial optimization focused on classical parameters: different host cells, inducer concentration, antibiotic selection pressure, induction timing relative to optical density, and culture medium amount. Recognizing the prohibitive cost of isopropyl- -D-1-thiogalactopyranoside (IPTG) at scale, this study hypothesized that lactose present in skimmed milk could function as a low-cost, biocompatible inducer of the lac operon. Comparative analyses between IPTG (0.1–0.2 mM) and skimmed milk (0.1–0.2% w/v) induction regimes revealed that skimmed milk at 0.1% w/v, administered at mid-log phase (OD 0.6), matched the induction efficiency of 0.2 mM IPTG, with no detectable compromise in protein solubility or activity. Under these conditions, large-scale cultures in LB medium achieved a 2.5-fold increase in melittin yield compared to standard IPTG induction. Our findings demonstrate that skimmed milk serves as a viable, economical alternative to chemical inducers, enabling sustainable high-yield expression of recombinant peptides in E. coli. This protocol is readily adaptable to other lac-regulated systems and holds significant promise for resource-limited research and industrial biomanufacturing. Keywords: Melittin, E. coli XL-1B, GST, IPTG, Skimmed milk, Overexpression CONTENTS 1. INTRODUCTION i 2. LITERATURE REVIEW iii 3. MATERIALS AND METHODS vi 3.1 Bacterial strains, plasmid, reagents used in this study. vi 3.1.1 Medium and growth condition. vi 3.2 Purification of Plasmid DNA. vi 3.3 Transformation of Recombinant MET into E. coli competent cell. vii 3.4 Over expression of Recombinant protein IPTG and skimmed milk. vii 3.5 Protein Estimation Assay. viii 3.5.1 Follin Lowry Assay 3.5.2 Preparation of BSA standard curve and quantification of protein concentration. viii 3.6 Protein Profiling x 3.6.1 SDS -Polyacrylamide Gel Electrophoresis x 4.Results xi 5. Conclusion. xix 6. References. xx 7. Plagiarism Report. xxi 1.INTRODUCTION The experimental work in recent days to produce Recombinant protein in Escherichia coli (E. coli) has been quite valuable in molecular biology as well as biotechnology because of its cost-effectiveness along with well-characterized genetics and also rapid growth rate. Since the bacterium can express heterologous proteins, it can host protein production ideally for enzymes, antibodies, and therapeutic peptides. Biomass concentrations over 100 g dry cell weight per liter are enabled by high-cell-density cultivation techniques.[1] Therefore, volumetric productivities are often in the gram-per-liter range when fed-batch and auto-induction strategies are in use. Additionally, vectors which are diverse, host strains which are diverse, and also fusion tags are available and do allow fine-tuning of expression parameters which maximizes both yield and solubility. Melittin has an amphipathic -helical structure with 26 amino acids making up 40–60% of honeybee (Apis mellifera) venom dry weight. It also exhibits a spectrum of bioactivities like strong antimicrobial, anti-inflammatory, antitumor, along with

antiviral effects.[2] A cationic N-terminus eases its insertion into lipid bilayers so this permeabilizes membranes plus lyses cells, attributes inspiring uses including novel antibiotic development to adjuvant therapies inside oncology plus virology.[3] Some difficult challenges face recombinant production within bacterial hosts because melittin has intrinsic cytolytic activity, also a small molecular weight near 2.9 kDa. Firstly, melittin's toxicity toward E. coli often causes poor cell viability coupled with expressing low levels unless someone sequesters it inside inclusion bodies or a fusion partner masks it. Secondly, its susceptibility toward those intracellular proteases can degrade both of the peptide with the fusion construct. To overcome these hurdles, fusion tags such as glutathione S-transferase (GST) have been employed to enhance solubility, protect against proteolysis, and facilitate affinity purification via glutathione-Sepharose chromatography. [4]. Despite these advances, existing methods frequently yield modest amounts of active melittin (1 mg/L), underscoring the need for further process optimization. Traditional induction of protein expression in E. coli utilizes (IPTG), a potent inducer of the lac operon. However, IPTG's high cost and potential toxicity at scale have prompted investigations into cheaper, biocompatible substitutes. Through this experimental study ,it serves certain integration of E.coli XL 1B strain infused with GST and skimmed milk at 0.1% (w/v) to result in melittin expression. We hypothesize that this combination will (i) protect host cells from melittin toxicity, (ii) sustain robust cell growth at high densities, and (iii) match or exceed the yields achieved with conventional IPTG induction, while drastically reducing production costs. Such an approach holds promise not only for melittin but also for other lac-regulated peptides and proteins, especially in resource-limited settings. 1. 2.LITERATURE REVIEW Melittin (MET), a peptide extract from the venom of Apis mellifera, the European Honey Bee is a widely researched ingredient for its cytolytic effects, as antibacterial, antiviral, anti-inflammatory and antitumor compound. MET is a thin linear cytolytic peptide made up of made up of 26 residues of amino acids in which the amino-terminal area is mainly hydrophobic, while the carboxy-terminal area is hydrophilic owing to the presence of a string of positively charged amino acids. MET has numerous biological, toxicological, pharmacodynamic, and pharmacological properties, including good cell-lipid membrane interactions, antifungal, antibacterial, antiviral, and potential anti-tumour activities. The need for new effective antimicrobial, anticancer and antiviral medicines have become a priority nowadays and new approaches should be introduced to the existing alternative therapeutic regimen. [6] Figure 1 Structure of Melittin [7] The melittin peptide, the predominant component of bee venom (40 48%, w/w), has been investigated substantially, and exhibits potent cytolytic and antimicrobial activities. [8] In the early 1970s, the effectivity of melittin against acid-fast microorganism Mycobacterium phlei was demonstrated In recent studies on clinically isolated M. tuberculosis and M. avium are shown to be effective. A class of parasitic bacteria , mollicutes those ate known for their absence of a cell wall phenotypically ,are sensitive to melittin . Even in past days melittin has been the basic for a number of novel AMP discoveries. many novel AMP discoveries. For example, pioneering work done by Bruce

Plagiarized Sources

vi 3.3 Transformation of Recombinant MET into E.
https://pubmed.ncbi.nlm.nih.gov/18997900

MATERIALS AND METHODS vi 3.1 Bacterial strains, plasmid, reagents used in this study.
https://pmc.ncbi.nlm.nih.gov/articles/PMC434458

coli utilizes (IPTG), a potent inducer of the lac operon.
https://pmc.ncbi.nlm.nih.gov/articles/PMC10639102